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Abstract

Recent investigations on the sesquiterpene-rich Amyris (*Amyris balsamifera* L.) and Siam-wood (*Fokienia hodginsii* L.) essential oils revealed significant arthropod repellency and toxicity responses. Amyris essential oil and one of its major components, elemol, were evaluated in laboratory bioassays and identified as effective mosquito repellents, specifically characterized by high levels of contact and minimal spatial repellency. Mosquito responses to catnip (*Nepeta cataria* L.) essential oil are characterized with high spatial activity, but lack significant contact repellency. Sampling within the staticair bioassay chamber with solid-phase microextraction provided measurements of the relative concentration and distribution of volatiles. These results supported the differences observed in repellency between essential oil treatments. Essential oil mixtures containing both spatial (catnip) and contact (Amyris) repellents were made and showed high levels of residual control via both modes of action. Siam-wood essential oil scored high in both spatial and contact efficacy against mosquitoes. Observations during this study included signs of toxicity. Two of the primary components of Siam-wood essential oil were tested for 24-hour house fly (*Musca domestica* L.) topical mortality. Transnerolidol and fokienol were found to possess similar insecticidal activity (topical LD50 values ranged from 0.17-0.21 μmol/fly). Amyris essential oil was selected for additional testing with brown dog ticks (*Rhipicephalus sanguineus*Latreille) in a 'barrier' repellency assay. Individuals were observed repeatedly avoiding and moving away from surfaces treated with Amyris essential oil.

Disciplines

Entomology | Other Pharmacology, Toxicology and Environmental Health | Parasitic Diseases | Plant Sciences

Comments

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Chapter 2

Amyris and Siam-wood Essential Oils: Insect Activity of Sesquiterpenes

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Recent investigations on the sesquiterpene-rich Amyris (*Amyris balsamifera* L.) and Siam-wood (*Fokienia hodginsii* L.) essential oils revealed significant arthropod repellency and toxicity responses. Amyris essential oil and one of its major components, elemol, were evaluated in laboratory bioassays and identified as effective mosquito repellents, specifically characterized by high levels of contact and minimal spatial repellency. Mosquito responses to catnip (*Nepeta cataria* L.) essential oil are characterized with high spatial activity, but lack significant contact repellency. Sampling within the static-air bioassay chamber with solid-phase microextraction provided measurements of the relative concentration and distribution of volatiles. These results supported the differences observed in repellency between essential oil treatments. Essential oil mixtures containing both spatial (catnip) and contact (Amyris) repellents were made and showed high levels of residual control via both modes of action. Siam-wood essential oil scored high in both spatial and contact efficacy against mosquitoes. Observations during this study included signs of toxicity. Two of the primary components of Siam-wood essential oil were tested for 24-hour house fly (*Musca domestica* L.) topical mortality. Trans-nerolidol and fokienol were found to possess similar insecticidal activity (topical LD_{50} values ranged from 0.17-0.21 μmol/fly). Amyris essential oil was selected for additional testing with brown dog ticks (*Rhipicephalus*

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sanguineus Latreille) in a 'barrier' repellency assay. Individuals were observed repeatedly avoiding and moving away from surfaces treated with Amyris essential oil.

Introduction

Nature holds a diversity of terpenoid structures, and the functionality of these compounds is still poorly understood. Only a small number actually serve a primary metabolic function (ex. carotenoids, sterols, etc.). In the 1970s, researchers started to identify other terpene bioactivities including toxicity, attraction, and repellency (*1*). The challenges today still include the characterization of terpene function, but also improvement of our understanding of their ecological roles. A variety of living organisms are known to utilize terpenes for coordinating antagonistic and beneficial interactions, such as inter- and intraspecific communication, and defense (*2*).

Terpenoid compounds are classified into groupings based on the number of isoprene units: hemiterpenes C5, monoterpenes C10, sesquiterpenes C15, diterpenes C20, sesterterpenes C25, triterpenes C30, tetraterpenes C40, and polyterpenes (terpene polymers). In plants, terpene biosynthesis pathways are either via the formation of a mevalonic acid intermediate or the pyruvate pathway. Mono-, sesqui-, and diterpenes are formed by continual addition of 5-carbon units, whereas other larger terpenes require joining of large carbon units, e.g. two sesquiterpenes to form a triterpene.

Bioactivity of Sesquiterpenes

Sesquiterpenes are produced in a number of plant families and appear in different concentrations in the essential oil composition. In many of these cases sesquiterpenoids make up only a small percentage of the essential oil blend, however there are examples of oils containing large amounts of these compounds with similar ring structures and specific functional groups. There is evidence of essential oils, and the actual plant tissues (heartwood, bark, leaves, etc.), containing sesquiterpenes with alcohol, aldehyde, and acid moieties, possessing high levels of insecticidal or repellent activity. The essential oil obtained from the bark of *Goniothalamus uvariodes* King, a small tree endemic to Borneo, is one example. Both the bark and leaves from this plant are used by several local groups including the Kedayan and Iban communities in Sarawak and the Sungai in Sabah as an insect repellent. The chemical constituents of the bark includes sufficient amounts of nerolidol (5.2%), α-eudesmol (5.6%), hedycaryol (13.6%), γ-eudesmol (16.0%), and β-eudesmol (31.5%) (*3*). These compounds and other closely related structures (farnesane, eudesmane, eremophilane, and elemene derivatives) appear in other reports detailing insect response to essential oils.

Several eudesmol isomers, and a eudesmane sesquiterpene acid and methyl ester derivatives were isolated from *Callitris glaucophylla* Thompson et Johnson and identified as termite repellents (*4*). The *Cryptomeria japonica* (L. f.) D. Don essential oil contains elemol as its major component (18.2%), and was recently identified as a repellent to silverfish (*5*). Another interesting study investigated the essential oil composition of *C. japonica* cultivars that varied in susceptibility to the *Cryptomeria* bark borer (*Semanstus japonicus* Lacordaire). Attractant and repellent responses of the *Cryptomeria* bark borer were used to assay select chemical components of the essential oils, and quantitative comparisons were made across the different cultivars. There were notable differences in the essential oil compositions of the resistant and susceptible cultivars, with the bark oils showing great diversity in structures and amounts of terpene hydrocarbons in particular, pinene (16-52%), limonene (7-12%), and δ-cadinene (4-8%). Many of the terpene hydrocarbons, e.g. β-pinene, camphene, sabinene, β-phellandrene, β-caryophyllene, and longifolene, were found to be attractants for the *Cryptomeria* bark borer. Four compounds were found to occur in significantly higher levels in the resistant cultivars and identified as repellents in the laboratory bioassay. These included three oxygenated sesquiterpenes α-terpineol, nerolidol, and β-eudesmol (*6*).

Callicarpenal and intermedeol were isolated from the American beautyberry bush (*Callicarpa americana* L.) and recently tested for insect activity. Researchers used a finger tip climbing assay and found both to be effective tick repellents. At an application rate of 155 nmole/cm^2 deer tick (*Ixodes scapularis* Say) nymphs were repelled 98 and 96%, respectively. These compounds were compared with commercial standard N,N-diethyl-m-toluamide (DEET) , and there was no significant difference with DEET (callicarpenal, EC_{50} 14.2 nmol/cm^2; intermedeol, EC_{50} 17.4 nmol/cm^2; DEET, EC_{50} 23.9 nmol/cm^2) (*7*).

Another collection of sesquiterpenoids from the heartwood of the Alaska yellow cedar (*Chamaecypars nootkatensis* D. Don), include nootkatone and valencene-13-ol. Both of these compounds were just as repellent to *I. scapularis* as DEET (nootkatone, RC_{50} 0.0458% wt/vol solution; valencene-13-ol, RC_{50} 0.0712% wt/vol solution; DEET, RC_{50} 0.0728% wt/vol solution) (*8*).

Amyris Essential Oil

West Indian sandalwood or Amyris oil (*Amyris balsamifera* L.) is produced from the heartwood of a small tree (3-6 m, 75-150 DBH) in the Rutaceae. Some of the identifying features of this tree include three to seven ovate, opposite and compound leaflets, white flowers in lateral clusters, and a black drupe fruit. Trees are described as having a smooth grayish bark, with a rounded crown of aromatic foliage. Its distribution is mostly limited to the Caribbean islands, but is also found in some South American countries. Amyris is also referred to as *bois chandelle* (candlewood) in Haiti, torchwood in Jamaica, *tigua* in Venezuela, but in the United States as Amyris, balsam amyris, or West Indian sandalwood. Interestingly, this species is not closely related to the other sandalwood (e.g. Indian or Australian sandalwoods), which are highly valued, wood-scented essential oils derived from trees in the Santalales. The sandalwood oils and other byproducts (including incense, pastes, and wood-carvings) have a rich history of being used in religious and social ceremonies.

Some other common uses for the Amyris heartwoods have included torches, firewood, fence posts, and ancient wood-carvings mosaics (*9*). This is not surprising considering the soft-quality of the heartwood and its use in carving. Also, there are studies citing the antimicrobial activity of Amyris extracts. Amyris essential oil is an effective inhibitor of *Klebsiella pneumonia* growth, and minimally effective against *Staphylococcus aureus* (gram-positive), *Escherichia coli* (gram-negative), and *Pseudomonas aeruginosa* (*10*). Such properties would no doubt be beneficial for maintaining the integrity of the wood in several of the uses listed above.

In most regions where Amyris is commercially grown, it is used for essential oil production. Steam distillation is estimated to yield 2-4%, depending on the portions of wood used. The essential oil is a viscous amber liquid composed mostly of oxygenated sesquiterpenes (80%) and sesquiterpene hydrocarbons (20%). Its woodsy scent is used in perfumery, soaps, and cosmetics and is also believed to be used by the cosmetic and perfume industries to dilute more expensive sandalwood oils such as that from East Indian sandalwood, *Santalum album* L. (*11*). There are also pharmaceutical and nutriceutical benefits from Amyris chemistries. Anti-mutagenic activity has been shown with β-eudesmol, one of the primary components. This compound suppressed SOS-inducing activity of furylfuramide, in addition to suppression of gene expression (ID_{50} 0.09 μmol/ml) in *Salmonella typhimurium* TA1535/pSK1002 with the furylfuramide mutagen 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide. Additional suppression activity was seen against the Trp-P-1 mutagen 3-amino-1,4-dimethyl-5H-pyridol[4,3-b]indole (*12*).

Previous studies in the Pesticide Toxicology Laboratory at Iowa State University, Ames, IA identified the repellent activity of Amyris essential oil against mosquitoes (*13*). Amyris was one of forty essential oils recently screened for repellency of *Aedes*, *Anopheles*, and *Culex* spp. mosquitoes using the human-bait technique (*14, 15*). The Amyris essential oil formulation provided a 480-minute protection period against *Anopheles* and *Culex* and 240 minutes for *Aedes*. Percentages of landing and biting mosquitoes reported was also low (*Anopheles*, 0% landing and biting; *Culex,* 0% landing and biting; *Aedes,* 9.6% landing and 0.8% biting). These levels were comparable to the Bayrepel and DEET formulations (*16*). Studies with Amyris essential oil as a potential mosquito larvicide were conducted using the yellow fever mosquito, (*Aedes aegypti* L.). With fresh preparations, researchers found 100% mortality of the mosquito larvae at 6 h following application, at a rate of 50 ppm (*17*). Efficacy following storage of this preparation showed that it was not effective after 1 week in a dark environment.

Siam–wood Essential Oil

Siam-wood (*Fokienia hodginsii* L.), which is also known as Vietnamese pemou, produces a highly prized oil from the heartwood in the Cupressaceae. These cypress trees are the only living species in the genus *Fokienia* and are adapted to growing at higher altitudes (600-1800m) in regions of Southern China, Northern Lao PDR, and Vietnam (*18*). Some of the people in these regions, such as the Greater Annamites, utilize the wood for housing and furniture construction. This is due to the longevity of the wood and its ability to handle many climatic factors and resist insect injury. The essential oil is extracted from the stumps and roots. Constituents of the essential oil were reexamined by Weyerstahl et al., and they found only sesquiterpenes. The major components identified were (E)-nerolidol (34.8%) and fokienol (25.7%); minor components were multiple cadinene isomers (6.5%), eudesmol isomers (7.4%), α-cadinol (1.9%) and dauca-8(14),11-dien-9-ol (3.1%) (*19*). There is limited literature available on the insect activity of Siam-wood extracts. Only one citation was found that mentioned that the wood is resistant to termites and moths (*19*).

The intent of this study was to characterize the bioactivity of two sesquiterpene-rich essential oils, Amyris and Siam-wood. In the initial screening trials, both oils showed evidence of repellency against a mosquito (*Ae. aegypti*). One area of particular interest was observation of residual repellency effects (including both contact and spatial repellency), which were supported by the relative concentration of volatiles measured inside the bioassay chambers. These essential oils were evaluated against actives contained in commercial natural products, and then incorporated into mixtures to test for improvements of natural product residual efficacy. The results of this study show that Amyris and Siam-wood significantly repel arthropods, are superior to other natural products in today's market, and could potentially be utilized to improve residual control in repellent formulations.

Materials and Methods

Mosquito Repellency Bioassay

Bioassays were conducted in a static-air apparatus (9 x 60-cm section of glass tubing) at a controlled temperature of 26°C. Yellow fever mosquitoes (*Aedes aegypti*), a Costa Rican strain, were from an established laboratory colony in the Iowa State University, Medical Entomology Laboratory, Ames, Iowa. Eggs were hatched in deoxygenated water, and larvae were fed Tetramin fish food (Melle, Germany). Pupae were sorted from the larvae and placed in paper cups with mesh lids until emergence. Newly emerged adults were fed a 10% (0.3 M) sucrose solution and aged for at least 5-days before testing. Incubator conditions were set at 60% relative humidity and held at 27°C. Only female mosquitoes were used in the testing.

Essential oils and mixtures included catnip (*Nepeta cataria* L.) oil, which was produced from a steam distillation in the laboratory (*20*). Amyris oil was purchased from Sigma Aldrich, St. Louis, Missouri; Siam-wood essential oil was purchased from Oshadhi, Petaluma, California. Elemol, a sesquiterpene found in both Amyris and Siam-wood essential oil, was purified from a crude commercial source (Augustus Oils, New Hampshire, England) using column chromatography techniques with silica gel. Several of the commercial repellent active compounds were available for purchase: DEET, citronella oil, 2-

undecanone, and cis/trans p-menthane-3,8-diol (Sigma Aldrich, St. Louis, Missouri).

Test solutions were made up in a carrier solvent (either acetone or hexane), applied to 9-cm diameter round filter papers (63.6 cm^2), and then the solvent was evaporated off prior to testing. The resulting rate of exposure was 78.6 $\mu g/cm^2$. Treated filter papers were placed inside the lids of 9-cm glass petri dishes, and the dishes were placed over the ends of the glass chamber. A group of 20 female mosquitoes were anaesthetized with CO_2 and introduced through a 2-cm hole drilled at the midpoint of the chamber. Mosquito distribution inside the static-air choice-test apparatus was observed over a total of 360-minutes. The experimental design was a completely randomized design using three replications of each treatment. Data generated by this study was used to examine two measures of mosquito repellency, **percentage (spatial) repellency** and **contact repellency**. Percentage repellency was calculated with the following formula to provide an indication of spatial repellency:

$$\text{Percentage Repellency} = ((\text{Number of Individuals in Untreated Half} - \text{Number of Individuals in Treated Half}) / 20) \times 100$$

Contact repellency was defined in this assay as 100% avoidance of the treated filter paper (no contact) throughout the 360 minute observation period. The resulting contact repellency was compared with control treatments, using Fisher's Exact Test.

Collection of Volatiles Using Solid-Phase Microextraction

Relative concentrations of volatiles were sampled inside the static-air glass apparatus used in the repellency bioassays. Test solutions were applied to filter papers at a rate of 78.6 $\mu g/cm^2$ and then enclosed in the system. Catnip essential oil, elemol, and DEET were selected, based on the differences in mosquito repellency (contact vs. spatial activity) observed in the previous bioassay. Temperature and light were held constant throughout the study. Solid-phase microextraction (SPME) field samplers containing a PDMS fiber (Supelco, St. Louis, Missouri) were conditioned in a GC inlet held at 250°C for 30 minutes before sampling. Holes were drilled in the center of equally-spaced quadrants of the static-air chamber and covered with a small amount of parafilm, to allow placement of the four SPME fibers in each volatile sampling replicate. Prior to the start of the study, static-air chambers were sampled with SPME fibers and identified a minimal level of background contamination.

SPME fibers were exposed inside the treated chambers for one of two 15-minute time periods; collection of volatiles was conducted immediately following treatment (0-15 min.), or 15 minutes after treatment (15-30 min.). Volatile samples were replicated three times for each test solution and time period. Relative concentrations of volatile samples were measured by GC-FID. Quantitative standards were made up for DEET (Sigma Aldrich), as well as elemol (≥ 80%), *Z,E*-nepetalactone (≥ 90%), and *E,Z*-nepetalactone (≥ 90%), which were purified in the laboratory by column chromatography. Theoretical vapor pressures were calculated using ACD/Lab Boiling Point software, Version 8.0.

House Fly Toxicity Test

Toxicity bioassays were performed with adult house flies (*Musca domestica* L.), from an established laboratory colony in the Iowa State University, Pesticide Toxicology Laboratory, Ames, IA. Individuals were chilled on a cooled surface and dosed with one µl of test solution on the ventral abdominal surface. Test solutions consisted of five different concentrations of the active ingredient in an acetone solvent along with an acetone-only control, dispensed using a topical applicator (Model PB-600, Hamilton Co., Inc., Whittier, California). Each concentration was applied to a population of 10 house flies and then placed in a screen-covered glass mason jar containing a cotton wick soaked in a saturated sucrose solution. Mortality was recorded after 24-hours. All treatments were replicated three times.

Tick Repellency Bioassay

Tick responses to candidate repellent essential oils and compounds were evaluated in a climbing arena. Positive controls consisted of DEET and a 20% pyrethrum solution (Sigma Aldrich). Brown dog ticks (*Rhipicephalus sanguineus* Latreille) were purchased from EL Lab, Soquel, California. Four individuals were placed in a glass Petri dish arena (area of 10.2 cm^2) surrounded by water, maintained at 23-24°C. In the center of the arena, a braided cotton wick was suspended. Treatments were made up as solutions in acetone and applied evenly across a “barrier”, designed at 2.54 cm from the bottom of the arena. The solvent was allowed to evaporate off the cotton wick (1-2 minutes) prior to the start of the test period. Ticks were allotted 60 minutes to search the arena and begin climbing behavior. The total number of ticks that attempted to climb the cotton wick was recorded. Individuals that passed the treated barrier were removed from the arena and recorded. If a tick approached the chemical barrier and either circled or turned around, the activity was noted and then the individual was allowed to continue movement in the arena until the 60 minutes had concluded. Five replications were completed for each treatment.

Results

Results for Amyris essential oil and for a mixture (1:1), containing a potent spatial repellent, catnip essential oil, are shown in Table 1. The difference between Amyris and catnip oils can be seen in the comparison of their percentage repellency values (measure of spatial repellency) and avoidance frequency (contact repellency). Amyris yielded a significant degree of spatial repellency compared to the control, but this percentage repellency value was lower than for the catnip oil. There was also a noticeable difference in avoidance frequency of Amyris and catnip. Amyris avoidance frequency accumulated over the 3-hour test period was 0.97, i.e. only 1 mosquito came in contact with the treated filter paper. The Amyris and catnip essential oil mixture resulted in significant levels of both spatial repellency and contact repellency.

Table 1. The 15-minute spatial repellency and 3-hour contact repellency of yellow fever mosquitoes (*Aedes aegypti*) exposed to 78.6 µg/cm^2 rate of Amyris and catnip essential oils and mixtures (1:1) in the static-air repellency chamber.

Treatment	*Percentage Repellency* [a]	*Std. Dev.*	*Avoidance Frequency*[c]	*Contact Rep.*[d] *(P value)*
Catnip Essential Oil	77.7*	14	0.19	0.218
Amyris Essential Oil	55.2*	23	0.97	<0.001
Catnip/Elemol Mixture	93.0*	11	0.83	<0.001
Catnip/Amyris Mixture	82.6*	20	0.94	<0.001
Elemol	63.6*	53	0.97	<0.001
Control	6.8	17	0.19	-

[a] Percentage repellency was determined at 15 minutes.

*Significantly different from control (α = 0.05) in LS means comparison.

[c] Avoidance frequency = average of mosquito contact repellency over 3-hour time period.

[d] Contact repellency = 100% of the individuals off treated surface.

Elemol makes up approximately 10% of the Amyris essential oil, along with a collection of other oxygenated sesquiterpenes (eudesmols, valerianol, etc.). Our laboratory has previously reported the mosquito repellent activity of elemol (*21*). When tested for spatial and contact mosquito repellency, elemol showed similar characteristics to its parent essential oil; significant spatial repellency that, on average is lower than catnip essential oil, but with higher levels of contact repellency. The elemol/catnip essential oil mixture provided a combination of highly significant spatial and contact repellencies.

The differences observed in spatial and contact repellency are also highlighted by the relative concentrations of these volatilized compounds inside the repellency bioassay chamber (Table 2). Higher amounts of *Z,E*- and *E,Z*-nepetalactone isomers (ratio in this sample of catnip essential oil was 75:25 *Z,E* / *E,Z*-nepetalctone) distributed quickly inside the repellency chamber, which would be expected of a good spatial repellent. Elemol and DEET, both highly significant contact repellents did not distribute as far, or as quickly as the nepetalactone isomers inside the chamber. Out of the four compounds tested, the lowest level of volatiles collected were in the DEET applications.

Table 2. Volatile collections (in nmol) of *Z,E* and *E,Z*-nepetalactone from catnip essential oil, elemol, and DEET (78.6 μg/cm² application rate) in the static-air glass apparatus using solid-phase microextraction with a PDMS fiber.

		Distance Away From Treated Surface			
Volatiles	*Time*	*8 cm*	*23 cm*	*38 cm*	*53 cm*
Z,E-nepetalactone* (V.P. = 1.75 mmHg)	15 min.	113	29	3	0
	30 min.	116	24	12	11
E,Z-nepetalactone* (V.P. = 1.75 mmHg)	15 min.	34	10	4	0
	30 min.	36	9	6	6
Elemol (V.P. = 0.24 mmHg)	15 min.	2	2	1	1
	30 min.	2	2	1	0
DEET (V.P. =0.58 mmHg)	15 min.	1	0	0	0
	30 min.	4	0	0	0

V.P. = vapor pressure (100°C) calculated by ACD Boiling Point software, Version 8.0.
*Isomer measurements made from surfaces treated with catnip essential oil.

Siam-wood essential oil was tested for efficacy in the short-term residual mosquito repellency bioassay. Results for these tests showed good residual spatial and contact repellency (Table 3).

Table 3. Spatial and contact repellency of yellow fever mosquitoes (*Aedes aegypti*) exposed to 78.6 μg/cm² application rate of Siam-wood and catnip essential oils and mixtures in the static-air repellency chamber.

	Percentage Repellency over Time				*Avoidance*	*Contact Rep.*[b]
Treatment	*1 hr*	*2 hr*	*3 hr*	*6 hr*	*Frequency*[a]	*(P value)*
Catnip Essential Oil	20.3	100% Mortality --------			0.25	0.217
Siam-wood Oil	82.2	92.9	96.3	72	1.00	<0.001
Catnip/Siam-wood Mixture (1:1)	74.1	74.1	100% Mortality		0.83	<0.001
Control	7.4	-14	-18	3.7	0	-

[a] Avoidance frequency = average of mosquito contact repellency over 3-hour time period.
[b] Contact repellency = 100% of the individuals off treated surface.

Some Siam-wood toxicity effects were observed in the repellency screening trials and motivated a house fly LD_{50} toxicity test with the two major components in its essential oil, fokienol and trans-nerolidol (Table 4).

Table 4. House fly 24-hour toxicity to trans-nerolidol and fokienol, two major components in Siam-wood essential oil.

Treatment	*LD_{50}*	*95% C. I.*
Nerolidol	0.17 μmol/fly	0.14 - 0.21
Fokienol	0.21 μmol/fly	0.12 - 0.34

Amyris (good contact repellent) and catnip (good spatial repellent) essential oils were selected for further testing against active components that are presently used in commercial topical mosquito products. Amyris and catnip essential oils, and p-menthane-3,8-diol were the only three actives to significantly differ in percentage repellency from the control in this study.

Table 5. Spatial and contact repellency tests with yellow fever mosquitoes (*Aedes aegypti*) to surfaces treated with active ingredients (78.6 μg/cm^2 application rate) of commercially available botanical-based repellent candidates and our targeted essential oils in a static-air repellency chamber.

		Average Percentage Repellency				
Product Name	*Active Ingredient*	*15 min.*	*30 min.*	*1 hr.*	Avoidance Frequency[a]	*Contact Rep.*[b] (P value)
OFF® Botanicals (SC Johnson)	p-menthane -3,8-diol	40	80*	78*	1.00	<0.001
BioUD	2-undecanone	41	19	16	0.33	0.093
SCENT OFF TWIST-ONS (ScentOff Corp)	Citronella Oil	52*	44	13	0.75	<0.001
Technical Grade	Catnip Oil	74*	59*	54*	0.66	0.001
Technical Grade	Amyris Oil	27	40	62*	0.83	<0.001
Solvent	Control	-11	-7.4	3.7	0	-

*Significantly different from control ($\alpha = 0.05$) in LS means comparison.

[a] Avoidance frequency = average of mosquito contact repellency over 1-hour time period.

[b] Contact repellency = 100% of the individuals off treated surface.

A small-scale 'barrier' test was used to study brown dog tick repellency. Amyris essential oil was evaluated against an untreated control, and two positive standards DEET and pyrethrum (20%).

The resulting tick climbing activity in the untreated control treatment was 65%. Amyris essential oil and DEET significantly repelled brown dog ticks. Out of 20 ticks that were exposed to Amyris essential oil, only one tick climbed past the Amyris essential oil barrier after repeatedly turning around and climbing down to the arena. No ticks crossed the DEET-treated barriers.

Table 6. Climbing activity of the brown dog tick (*Rhipicephalus sanguineus*) when exposed to barrier-treated surfaces.

Treatment	*Application Rate*	*Percentage Climbing Past Barrier*	*Std. Dev.*
Amyris Essential Oil	1.25 mg/cm^2	5*	11.2
DEET	1.25 mg/cm^2	0*	0
Pyrethrum	1.25 mg/cm^2	40	22.3
Control	-	65	22.3

*Significantly different from control ($\alpha = 0.05$) in LS means comparison.

Conclusions

Plant essential oils are a rich source of sesquiterpenes that can both affect insect behavior and cause mortality. In particular, this study focused on essential oils that contain a select number of closely related sesquiterpenes. Amyris and Siam-wood essential oils were both tested and identified as effective mosquito repellents in a laboratory bioassay. Amyris essential oil was also an effective barrier against brown dog ticks. The majority of these essential oil compositions include oxygenated derivatives of farnesane, eudesmane, eremophilane, and elemane sesquiterpenes. Some of these also are present as primary components of other essential oils (American beautyberry bush, Alaska yellow cedar, etc.) that posses repellent properties. However, interpretation of the sesquiterpene functionality is often times confounded by differences of chirality. One such example is the study of gossypol (+) and (-) enantiomers, found in the cotton plant. These enantiomers have been shown to differ in toxicity to herbivores and pathogens (*22, 23*).

The mosquito laboratory assay in this study allowed for differentiation between contact and spatial repellent activities. High percentage repellency values were observed from mosquitoes exposed to catnip essential oil. The majority of individuals preferred to stay > 1 ft away from the treated surface, representing a significant level of spatial repellency when compared to the control. This observed behavior was not surprising considering the relative concentration of the *Z,E*:*E,Z*-nepetalactone isomers that distributed inside the static-air chamber. Spatial repellency of Amyris essential oil, although lower than catnip, was significantly different from the control treatment and comparable with actives contained in commercial mosquito repellents. Contact repellency, which was measured by cumulative observations of mosquito avoidance of the treated surfaces, was highly significant with Amyris oil. Throughout the 3-hour test period, only one individual came in contact with the treated surface. Similar results of high contact and minimal spatial repellency were seen when testing efficacy of elemol. Relative volatility of elemol, one of the primary components of the Amyris essential oil, was also sampled inside the static-air chamber and did not distribute throughout the chamber as quickly as the nepetalactone isomers. These results show that a chemical's volatility can be an important factor for spatial repellency, affecting the concentration that reaches the insect (*24, 25*). Interestingly, this significant spatial repellency did not always align with effective contact repellency. In the catnip trials there were several mosquitoes that came in contact with treated surfaces and there was no significant difference when compared to the control. These results are consistent with previous studies that have noted the minimal residual effects of catnip essential oil (*21*). This end result is similar to residual effects often observed with many of the first-generation natural repellents. Fradin and Day (*26*) evaluated the protection time of several commercially available repellent formulations, including citronella, peppermint oil, cedar oil, lemongrass oil, and geranium oil. On average, these products provided from 1 to 60 min. of protection whereas DEET formulations scored in a range of 200 to 360 min.

Comparison of catnip and Amyris essential oil shows that volatility isn't the only factor contributing to the repellent activity. Studies that explored the activity of vetiver essential oil found that the individual components' volatility was inversely related to termite repellency (*27*). Based on the characteristic differences in mosquito repellent activity, a mixture containing catnip essential oil (which provided good spatial activity) and the sesquiterpene-rich Amyris essential oil (good contact repellency) was tested. This mixture gave excellent mosquito repellency values via both contact and spatial modes of action. One of the major components in Amyris essential oil, elemol, was also made up in a mixture with catnip essential oil and found effective.

Amyris essential oil was selected for further testing against the brown dog tick. In a climbing arena, individuals that were exposed to an Amyris essential oil barrier would not cross it and frequently avoided contact. These findings were compared with results from a DEET-treated barrier, which successfully prevented ticks from climbing past the chemical barrier. A pyrethrum solution was also tested, but did not significantly prevent ticks from climbing past the barrier.

Siam-wood oil, which contains nerolidol and fokeinol, was also tested for efficacy and evaluated in a mixture with catnip essential oil. Results for these tests showed high levels of both spatial and contact mosquito repellency. Additionally, some mosquito mortality was observed at the concentrations tested inside the static-air chamber. The two major components of Siam-wood were identified as significantly toxic to house flies. To our knowledge, this is the first documented report of insect repellency and toxicological investigation of Siam-wood essential oil.

These findings highlight the potential use of catnip, Amyris, and Siam-wood essential oils for arthropod management. Although the specific repellency mode action of these oils appears to differ in terms of contact and spatial activity, formulated combinations of these did show improvement in a controlled laboratory setting. It is possible that similar mixtures might increase protection efficacy of other natural products.

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